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In the Specification

The specification is amended as follows:

On page 2, lines 21 to 24:

B1 --In general, the invention features substantially pure fucosyltransferase DNA or protein obtained from a plant. In a related aspect, the invention features a fragment or analog polypeptide including an amino acid sequence substantially identical to the sequences shown in ~~Tables 2, 5 and 6~~ SEQ ID NOs: 1, 5 and 7.--

On page 2, lines 25 to 31:

B2 --In another related aspect the invention features substantially pure DNA having a sequence substantially identical to the nucleotide sequence shown in ~~Tables 3-14~~ SEQ ID NOs: 2, 3, 4, 6, 8, 9, 10, 11, 12, 13, 14, and 15. In preferred embodiments, such DNA is cDNA or is genomic DNA. In related aspects, the invention also features a vector and a cell (e.g., a plant) which includes such substantially pure DNA. In various preferred embodiments, the vector-containing cell is a prokaryotic cell, for example, E. coli or Agrobacterium, or, more preferably, a plant cell.--

On page 2, line 32 to page 3, line 1:

B3 --In yet another related aspect, the invention features a method of fucosylating a polypeptide in vivo involving: (a) providing a cell containing the ~~fucosyl~~

~~transferase~~ fucosyltransferase DNA of the invention positioned for expression in the cell; and (b) culturing the transformed cell under conditions for expressing the DNA, resulting in the fucosylation of the protein. In preferred embodiments, fucosylation occurs in a plant cell-.

On page 3, lines 2 to 5:

-In another aspect, the invention features a recombinant polypeptide fucosylated using a cell expressing DNA which is substantially identical to the nucleotide sequence shown in ~~Tables 3-14~~ SEQ ID NOs:2, 3, 4, 6, 8, 9, 10, 11, 12, 13, 14, and 15. In still other preferred embodiments, the polypeptide is further fucosylated using one or more ~~fucosyl transferases~~ fucosyltransferases.--

Page 3, lines 6 to 12:

The present invention further includes multiple types of DNA constructs including (1) "sense" constructs encoding proteins, which can increase the expression of ~~fucoysltransferases~~ fucosyltransferases in plant species and (2) "antisense" constructs containing DNA, which can be used to produce antisense RNA in to reduce expression of ~~fucosyltransfereases~~ fucosyltransferases in plants. Optimal amounts of antisense RNA in transgenic plants will selectively inhibit the expression of genes in these plants which are involved in the ~~fucoylsation~~

B5

fucosylation of xyloglucans.--

Page 3, lines 21 to 31:

-In one embodiment, the first category of DNA constructs include: a promoter selected from but not limited to constitutive, tissue-specific, cell-type specific, seed-specific, flower-specific, fruit-specific, epidermis-specific promoters, a promoter specific to cell layers adjacent to the epidermis or a promoter specific to photosynthetically active plant tissues, which

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functions in plant cells to cause the production of an RNA sequence. In this embodiment, the DNA coding region sequences that encode proteins which can be used to increase the activity of plant ~~fucosyl transferases~~ fucosyltransferases in transgenic plants. The DNA coding region will further include a region 3' to the coding regions the 3' non-translated region which functions in plant cells to cause the addition of polyadenylate nucleotides to the 3' end of the RNA sequence promoter.--

Page 3, line 32 to page 4, line 3:

-In another embodiment, a second category of DNA construct will include a constitutive promoter, seed-specific, flower-specific, fruit-specific, epidermis-

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specific promoter, a promoter specific to cell layers adjacent to the epidermis or a promoter specific to photosynthetically active plant tissues, which functions in plant cells to cause the production of an RNA

sequence. The DNA construct will also include DNA sequences which can produce antisense RNA molecules. These RNA molecules can selectively inhibit the accumulation of transcripts encoding proteins which encode plant ~~fucosyl transferases~~ fucosyltransferases.--

Page 4, lines 4 to 18:

--In accordance with another aspect of the present invention, there is provided a method of producing genetically transformed plants which express a gene or genes involved in ~~fucosyl transferase~~ fucosyltransferase activity. In this method, a recombinant, double-stranded DNA molecule is incorporated into the genome of a plant cell. In this embodiment, the DNA sequence will include a promoter which functions in plant cells to cause the production of an RNA sequence in flowers, seeds, fruit or other plant tissues. In addition, the sequence will include a DNA coding sequence encoding proteins involved in ~~fucosyl transferase~~ fucosyltransferase activity in plants. Alternatively, the sequence will be a template to the synthesis of antisense RNA inhibiting the development of these structures. The DNA sequence will also include a 3' non-translated region which functions in plant cells to cause the addition of polyadenylate nucleotides to the 3' end of the RNA sequences. The method also includes obtaining transformed plant cells and regenerating from the

transformed plant cells genetically transformed plants. The transformed plant cells may be used to overproduce in cell culture the fucosylated xyloglucans.--

Page 4, line 24:

-BRIEF DESCRIPTION OF THE ~~DRAWINGS~~ DRAWING

~~This invention will be better understood by reference to the figures, in which.~~

Page 4, lines 27 to page 5, line 17:

~~**Figure 1** shows the biochemical purification of xyloglucan-specific fucosyltransferase from pea. Figure 1A shows a silver-stained SDS-PAGE gels showing protein profiles from carbonate-washed pea epicotyl extract, peak fractions from a GDP-agarose and size exclusion column, and the entire profile of all fractions from an anion exchange column. Figure 1B shows a xyloglucan-specific fucosyltransferase activity, in nKat (= nMoles of substrate incorporated into product per second), for each fraction of the anion exchange column eluate.~~

~~**Figure 2** shows confirmation of Arabidopis fucosyltransferase (Ftase) activity. Figure 2A shows that Anti-AtFT1 polyclonal antibodies recognize an approximately 62 kDa polypeptide in solubilized membrane proteins of Arabidopsis, but not pea. Lane 1, antigen (50 ng); Lane 2, Arabidopsis (40:1), Lane 3, pea (40:1.) Left, Western blot. Right, Commassie staining of membrane showing protein profiles. Figure 2B shows that~~

~~anti-FTase antibodies immunoprecipitate more XG-specific FTase activity than an equal volume of preimmune serum. Shown is FTase activity, in nanokats, of precipitated pellets that were subsequently subjected to FTase activity assays. This is an example similar to results seen in seven different replicates. Figure 2C shows that a full-length AtFTase which was expressed in a COS cell line shows XG-specific FTase activity. Activity in nKat is shown in the presence or absence of tamarind XG for untransformed Cos-7 cells, cells transformed with vector DNA (pCDNA3), cells transformed with vector containing AtFT1 (pCDNA-XGFT), or solubilized pea Golgi vesicles.~~

B9 Amt

~~**Figure 3** shows a hydrophobicity plot of AtFT1.~~

Page 5, line 19:

~~**Figure 4**~~ **Figure 1** shows a diagram of plasmid pMEN020.

Page 5, lines 32 to 34:

Xyloglucan fucosyltransferase: Xyloglucan fucosyltransferase (XG ~~Ftase~~ FTase) is an enzyme that fucosylates xyloglucan by adding a fucose residue to xyloglucan.

B10

Page 8, line 29 to Page 9, line 8:

A biochemical approach was utilized to purify sufficient quantities of xyloglucan fucosyltransferases from pea epicotyls. Pea microsomes were prepared as follows: 2 cm segments, excised just below the apical

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hook, of etiolated Pisum sativum, cv Alaska were collected and homogenized in 1.5 volumes buffer (50 mM Hepes pH 7.5, 1 mM EDTA pH 8.0, 0.4 M sucrose, 1 mM DTT, 0.1 mM PMSF, 1:g/mL each aprotinin, leupeptin, and pepstatin.) The homogenate was filtered, centrifuged at 2,000 x g for 15 minutes, and the ~~supernatent~~ supernatant was centrifuged at 100,000 x g for 1 hour. The resulting pellets were washed and homogenized in the presence of 0.1 M Na_2CO_3 to strip away peripheral membrane proteins (Y. FuJiki, A. L. Hubbard, S. Fowler, P. B. Lazarow, J. Cell Biol. 93, 97 (1982).) The suspension was centrifuged at 100,000 x g for 1 hour and the resulting pellets were washed and resuspended in buffer (50 MM Pipes-KOH pH 6.2, 20% glycerol, 1 mM EDTA, 1 mM DTT, 0.1 M PMSF, 1 :g/mL each aprotinin, leupeptin, and pepstatin.) The suspension was homogenized, mixed with TritonX-100 to a final volume of 0.8%, and stirred for 1-2 h to solubilize membrane proteins. The suspension was centrifuged a final time at 100,000 x g for 1 h and the ~~supernatent~~ supernatant was collected and saved.--

Page 9, line 20:

-~~**Fucosyl Transferase**~~ **Fucosyltransferase Assay**-

Page 9, lines 21 to 25:

-A specific assay for ~~fucosyl transferase~~ fucosyltransferase was developed using tamarind or nasturtium storage xyloglucan, which naturally lack

fucosyl residues, as an acceptor and radiolabeled GDP-fucose as a donor [V. Farkas, G. Maclachlan, *Arch. Biochem. Biophys.* **264**, 48 (1988). A. Camirand, D. Brummell, G. Maclachlan, *Plant Physiol.* **84**, 753 (1987)].-

B13 cont

Page 9, line 28 to page 10, line 6:

-To confirm that the purified pea protein synthesizes an alpha-1,2 fucose:galactose linkage, carbohydrate analysis was performed on the product resulting from in vitro fucosylation of tamarind xyloglucan by purified FTase. Carbohydrate linkage analysis of tamarind xyloglucan before (tamarind xg) and after (fucosylated xg) incubation with purified pea FTase. Samples were incubated at room temperature for 20 minutes (for immunoprecipitation samples) or 30 minutes (for protein purification samples) with 25 mM Pipes-KOH pH 6.2, 0.5 mg/mL tamarind xyloglucan, 0.05% ^{3}H GDP-fucose (3.7 mBq/mL, 300 GBq/mM, NEN, Boston, MA). Most assays also contained 50:M non-radiolabeled GDP-fucose to provide a quantitative measurement of enzyme activity. Assays of immunoprecipitation samples also contained 5 mM $MgCl_2$. Reactions were precipitated using 70% ethanol and ^{3}H incorporation was measured by scintillation counting. The amount of fucose incorporated into the product was used to calculate activity in nanokats (nKat - nMoles ~~substrated~~ substrate incorporated into product per second.) The ~~resullts~~ results are shown in Table 1.-

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Page 10, lines 23 to 27:

-It was possible to purify XG FTase 1400-fold by the end of the size exclusive ~~chromatograpy~~ chromatography step resulting in a total of 0.05 mg protein containing 70 nKat XG FTase activity. After biochemical purification and subsequent assay analysis, two polypeptides of approximately 65 kDa and 60 kDa in size were observed to co-purify repeatedly with XG FTase activity (Figure 1~~.~~).-

B15

Page 10, line 28 to page 11, line 3:

-Limited peptide sequence was obtained from both proteins. Proteins in size exclusion column eluate fractions containing peak amounts of FTase activity were concentrated using a Millipore 4 mL 10 kDa concentrator and separated by electrophoresis. After brief staining with Coomassie and destaining the separated proteins were excised, rinsed in 505 acetonitrile, stored at -80°C and sent to Harvard ~~Microchernistry~~ Microchemistry (Cambridge, MA) for tryptic peptide sequencing. Six peptide sequences were obtained: VFGFLGR (SEQ ID NO:16), YLLHPTNNVWGLVVR (SEQ ID NO:17), AVLITSLSSGYFEK (SEQ ID NO:18), YYDAYLAK (SEQ ID NO:19), LLGGLLADGFDEK (SEQ ID NO:20), and ESILPDVNR (SEQ ID NO:21).-

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Page 11, line 5:

B17 -**~~Arabididopsis~~ Arabidopsis EST Identification-**

Page 11, lines 6 to 11:

-Using these peptides as a query in the Blastp program identified an Arabidopsis EST, 191A6T7, which contained four out of six peptides in a deduced translation of a potential ORF. The 65kDa peptide was identified as a homolog of BiP, the usually ER-localized molecular ~~chaparone~~ chaperone. It is possible that this ~~chaparone~~ chaperone co-purified with FTase activity as an artifact and prevented the denaturation of the FTase during purification, though this has not been confirmed.-

B18

Page 12, lines 10 to 23:

-The portion of AtFT1 encoding aa 73 to 566 was PCR-amplified using appropriate primers and cloned into the pET28a expression vector (Novagen, Madison WI.) The resulting insoluble fusion protein was purified by washing four times with 1% Triton X-100, 50 mM Hepes-KOH pH 7.6, 10 MM $MgCl_2$ and one time with 25 mM Hepes-KOH pH 7.0, 8 M urea. Te pellet was resuspended in 6 M guanidine-HCl and protein was precipitated from the ~~supernatent~~ supernatant with 10% TCA. The protein was emulsified with Titermax ~~adjuvent~~ adjuvant (CytRx Corporation, Norcross, GA) and injected into a rabbit. For western blotting, 40:1 of carbonate-washed solubilized protein from pea and Arabidopsis and 50 ng of purified antigen were separated by SDS-PAGE and electroblotted. Anti-AtFT1 Abs (1:5000) were used for western blotting. Goat-antirabbit antibodies conjugated

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to horseradish peroxidase was used as a secondary antibody. Signals were detected by the enhanced chemiluminescence method (Pierce, Rockford, IL~~.~~). Anti-AtFT1 polyclonal antibodies recognize an approximately 62 kDa polypeptide in solubilized membrane proteins of Arabidopsis, but not pea. Membranes were then stained with Coomassie blue to detect protein.-

B19 Cont

Page 12, line 26 to page 13, line 3:

-For immunoprecipitations, solid NaCl was added to carbonate-washed solubilized Arabidopsis protein to a final concentration of 200 mM. The Arabidopsis protein was precleared by incubation with 1/10 volume of 50% slurry of protein A sepharose beads (Pharmacia) in buffer A (25 mM Pipes-KOH pH 7.5, 50 MM NaCl, 2 mM EDTA pH 8.0.) The resulting ~~supernatents~~ supernatants were incubated with 50:1 of immune or preimmune anti-AtFT1 serum for 1 h. 1/5 volume of protein A sepharose slurry was added to precipitate the antigen-antibody complexes and the samples were incubated for an additional 3 hours with rocking at 4 degrees C. Samples were then centrifuged, washed five times in buffer A containing 1% Triton X-100 and two times in buffer A without detergent. The pellets were resuspended in buffer A to a final volume of 120:1 and assayed for AtFTase activity as described above.-

B20

Page 13, lines 3 to 7:

-The immunoprecipitated proteins were then

B21

B21 Cont

assayed for XG FTase activity. More FTase activity was correlated with pellets derived from immunoprecipitation reactions using immune antiserum rather than preimmune serum, thereby indicating that the Arabidopsis clone encodes a xyloglucan-specific FTase ~~(Figure 2)~~.

Page 13, lines 10 to 28:

-Cos-7 cells were grown on 100 mm plates in DMEM-10% Fetal Bovine Serum. Cells were transfected with different plasmids using Lipofectamine™ reagent (Life Technologies) following the manufacturer's instructions using 9 :g of DNA and 72 :g of Lipofectamine. Cells were incubated for 24 hours in the medium containing DNA-Lipofectamine without Fetal Bovine Serum. The medium was changed to DMEM-10% Fetal Bovine Serum and incubated for another 48 hours. The cells were scraped off the dish in

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0.25 M sucrose, 10 mM Tris-HCl pH 7.5 and 0.4% CHAPS. XG-FTase activity was measured using 50 :g of protein in the absence (-XG) or presence (+XG) of 100 :g tamarind xyloglucan. The incubation was ~~carred~~ carried out in a volume of 0.1 mL in the presence of 1 :M GDP-Fuc; (93,000 dpm), 10 mM ~~MnCl2~~ $MnCl_2$, 20 mM Hepes pH 7.0, 0.05% Triton X-100 at 25°C for 90 min. The reaction was halted by adding ethanol to a final concentration of 70%. Samples were incubated at 4°C and filtered through 1.5 :m glass fiber filters. The filters were washed with 70% ethanol containing 1 mM EDTA. The filters were dried and

radioactivity determined by liquid scintillation. A control using pea Golgi vesicles was carried out in parallel. The results indicate that AtFT1 expressed in a COS cell line showed in vitro FTase activity that was 41 times higher than COS cells transformed with an empty vector and 1.4 times higher than solubilized pea Golgi vesicles ~~(Figure 2)~~.-

B22 Amt

Page 13, line 33 to page 14, line 5:

-Analysis of AtFT1 indicates that, while it has some structural characteristics common to other fucosyltransferases, it is quite divergent at the amino acid sequence level. Hydrophobicity plots predict that there may be a N-terminal transmembrane signal anchor sequence ~~(see Figure 3)~~. In vitro translation in the presence of canine pancreatic microsomes followed by carbonate washing of the products indicates that the AtFT1 translation product is a membrane protein (data not shown~~.)~~). As with other glycosyltransferases, the C-terminal region is predicted to be largely hydrophilic.-

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Page 16, lines 6 to 10:

Once identified, ~~fucosyl transferase~~ fucosyltransferase genes can be expressed in a variety of cells including plant cells, yeasts, fungi, bacterial cells and mammalian cells. A wide variety of plants can be transformed to express ~~fucosyl transferase~~ fucosyltransferase genes and genes related to ~~fucosyl~~

B24

B24 Cancel ~~transferase~~ fucosyltransferase in order to regulate plant ~~carbohdrate~~ carbohydrate glycosylation.--

Page 17, lines 5 to 26:

-A variety of expression vectors can be used to transfer the gene encoding plant ~~fucosyl transferase~~ fucosyltransferase activity as well as the desired promoters and regulatory proteins into a plant. Examples include but not limited to those derived from a Ti plasmid of *Agrobacterium tumefaciens*, as well as those disclosed by Herrera-Estrella, L., et al., Nature **303**: 209 (1983), Bevan, M., Nucl. Acids Res. **12**:8711-8721 (1984), Klee, H. J., Bio/Technology **3**: 637-642 (1985), and EPO Publication 120,516 (Schilperoort et al.) for dicotyledonous plants. Alternatively, non-Ti vectors can be used to transfer the DNA constructs of this invention into ~~monotyledonous~~ monocotyledonous plants and plant cells by using free DNA delivery techniques. Such methods may involve, for example, the use of liposomes, electroporation, microprojectile bombardment, silicon carbide ~~wiskers~~ whiskers, viruses and pollen. By using these methods transgenic plants such as wheat, rice (Christou, P., Bio/Technology **9**:957-962 (1991)) and corn (Gordon-Kamm, W., Plant Cell **2**:603-618 (1990)) are produced. An immature embryo can also be a good target tissue for monocots for direct DNA delivery techniques by using the particle gun (Weeks, T. et al., Plant Physiol.

B25

102:1077-1084 (1993); Vasil, V., Bio/Technology **10**:667-674 (1993); Wan, Y. and Lemeaux, P., Plant Physiol. **104**:37-48 (1994), and for *Agrobacterium*-mediated DNA transfer (Hiei et al., Plant J. **6**:271-282 (1994); Rashid et al., Plant Cell Rep. **15**:727-730 (1996); Dong, J., et al., Mol. Breeding **2**:267-276 (1996); Aldemita, R. and Hodges, T., Planta **199**:612-617 (1996); Ishida et al., Nature Biotech. **14**:745-750 (1996)). In addition, plasmid pMEN020 is described in ~~Figure 4~~ Figure 1.--

Page 18, lines 17 to 24:

-Fucoxyloglucan (XG) is the major hemicellulosic polysaccharide in the primary cell wall of dicots. Monocots have small ~~quantititties~~ quantities of XG, but it seems to be much less important in all monocots including grasses. XG has a backbone of beta-1,4 linked glucosyl ~~reisdues~~ residues, with three out of every four residues ~~substitituted~~ substituted with xylose in a regular repeat, i.e. three substituted followed by one free. Approximately one out of six of the xylosyl residues is further substituted with galactose and on the two position of ~~galatose~~ galactose is an alpha linked fucosyl residue. Thus, the fucose is a peripheral sugar in this polymer. However, the fucose has been postulated to be very important in determining the conformation of the polysaccharide, including controlling interactions of XG with cellulose. Thus, the presence of fucose may be

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important for the function of this polysaccharide.-

Page 18, lines 29 to 33:

-Since XG is the major hemicellulosic polysaccharides in dicots, including many important weeds, but not very abundant in the cell walls of monocots, including corn, wheat, ~~rice,barley~~ rice, barley, etc., inhibitors of XG synthesis may be valuable herbicides. Inhibitors include specific inhibitors of the enzyme itself and antisense constructs for inhibiting expression of the protein.-

B27

Delete Page 22 to 34 (Tables 2-14).